

THE EFFECTS OF PAVLOVIAN CONDITIONING IN TWO DISCRETE
ENVIRONMENTS

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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December 2015

Major Subject: Biomedical Sciences

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ABSTRACT

The purpose of this literature review is to examine whether Pavlovian eye blink conditioning in the rabbit consists solely of a specific motor reflex or involves other extracerebellar control systems. Castiglioni et al. (2009) utilized a transfer design in which retention of learning in different environments and the expression of the original learning was not limited to a single motor reflex measure. Subjects were trained in a traditional Pavlovian stock to differentiate between an S+ signal of a brief tone paired with an unconditioned stimulus (US) of cutaneous shock. Then, subjects were tested for what they had learned in a different environment.

The results showed that the presentation of the S+ produced completely different behavior in the open environment as compared to the Pavlovian stock. In the restricted environment of the Pavlovian stock, presentation of the S+ produced eye blink responses without any additional motor or emotional reactions. In the transfer environment, reaction to the S+ produced: (a) a prolonged disruption of ongoing behavior, (b) a wide range of complex emotional responses, and (c) a complete absence of eye blink response. Reaction to the S- in the Pavlovian stock or in the open environment, did not elicit an eye blink response. These findings provide strong evidence that Pavlovian conditioning is not just a specific motor reflex, but instead involves a difference in meaning and significance of the S+ for the subjects when presented in two discrete environments. This is observed in the subjects' different response patterns which unmasked a significant emotional component of the conditioning. This lead to the

conclusion that nictitating membrane (NM) conditioning consists of more than an invariant, discrete cerebellar oculomotor reflex. The clear emotional component to the conditioning indicates the involvement of extracerebellar control mechanisms, potentially a widespread cerebral network in addition to the microcerebellar control of the specific motor reflex, a contradiction to current neuromodels.

DEDICATION

To my parents and brother.

ACKNOWLEDGEMENTS

I would like to thank my committee chairs, Drs. Steele-Russell and Welsh, and my committee members, Dr. Pine, and Dr. Tizard for all their insight, guidance and support throughout my higher education journey.

A special thank you to Dr. Tomasz Werka at the Nencki Institute of Experimental biology, Dr. Joseph Castiglioni, and Murat Russell from the Sketch Recognition Lab of Texas A&M University.

NOMENCLATURE

CPT	continuous performance task
NM	nictitating membrane
S+	conditioned stimulus, reinforced with shock
S-	unreinforced stimulus
SST	sensory salience test
ITI	intertrial interval
US	unconditioned stimulus

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INTRODUCTION

With current advancements in medicine and healthcare, the average lifespan grows steadily longer. Concurrent with this is a steady increase in diagnoses of ocular disorders and visual impairment which are common in the elderly. Similarly, the growth rate of chronic and comorbid conditions related to increased age increases every year. This is in part due to the spread of the obesity epidemic, particularly in first world nations. Currently, 66% of adults in the United States are classified as overweight to obese (Wang & Beydoun, 2007). The obesity epidemic in particular, provides a strong addition to visual impairment through comorbid diabetic retinopathy, glaucoma, and cataract - all common complication of diabetes mellitus. A CDC study revealed that elderly people with visual impairment experienced a higher comorbidity with other conditions than elderly people without visual impairment (Crews, Jones, & Kim, 2006).

In vision research, animal models are a critical part of experiments to investigate disease mechanisms, their progression, and the development of treatments. The majority of vision studies are conducted with the rat optic model (Williams, 2002). Rat models are used to study various visual aspects, from eye development to visual disorders. These rat models are popular due to their low cost of purchase, maintenance, and upkeep, their sparse living space requirements, and quick gestation period compared to other non-rodent animals. However, the accuracy of rat optic models is suspect, as the limitations of their vision is far inferior to other mammals (Bawa et al., 2013; Zhou, Bedggood & Metha 2012) and calls into question the ability of the rat optic model research to

accurately act as a model for human vision. Rat vision has been called poor since as early as 1903 (Watson, 1903). A more suitable alternative to the rat model would be an animal with superior visual abilities and in possession of visual acuity. The rabbit is such a suitable subject. Their superior visual capabilities compared to the rat are outlined in Table 1 below (I. Steele-Russell, Functional Neuroanatomy, 1/3/2013).

Table 1. *Comparative visual anatomy.*

	MONKEY	CAT	RABBIT	RAT
VISION	diurnal	crepuscular	crepuscular	nocturnal
EYE POSITION	FRONTAL	FRONTAL	LATERAL	LATERAL
RETINAL TYPE	FOVEAL	AREA CENTRALIS STREAK	AREA CENTRALIS STREAK	CONCENTRIC GRADIENT
BINOCULAR FIELD	130°	100°	22°	50°
PURSUIT MOVEMENTS	PRESENT	PRESENT	PRESENT	NONE
VERGENCE MOVEMENTS	35°	100°	22°	50°
OPTIC NERVE FIBERS	1,210,000	193,000	397,000	95,000
PERCENTAGE CROSSED	50%	40%	10%	10%
IPSILATERAL FIBERS	650,000	77,200	39,700	9,500
GANGLION CELLS /°	55	21	17	2
RESOLUTION (minutes)	1	10	19	not known
ACUITY (cycles/°)	64Hz	6Hz	4Hz	0.3Hz

As seen in Table 1, rabbits' superior visual capabilities lie in their nature and anatomically in their larger optic nerve. Rabbits have crepuscular vision whereas rats are nocturnal creatures. Low light and low visibility at night, their active periods, suggest

rats rely on an integration of many other developed senses to navigate, such as vibrissal input, hearing, and smell (Favaro et al., 2011; Cardenas, Lamprea & Morato 2001; Carvell & Simons 1990) in conjunction with their visual capabilities. Rats have on average, 0.3 cycles/° acuity, which is especially low when compared to the average human or primate acuity of 64 cycles/° (Hughes, 1977). High visual acuity is necessary in rabbits to locate predators, potential threats, and sustenance.

Visual pursuit movements has been confirmed in rabbits and studied by Collewijn (1977); these voluntary shifts in gaze are designed to keep the visual stimulus focused on the fovea of the retina - the only area of the retina capable of high visual acuity. Rats lack the retinal structure analogous to a fovea (Euler & Wässle, 1995), this drastically diminishing their visual acuity capabilities. Research has confirmed this low visual acuity in rats (Douglas et al., 2005) and also their lack of any significant capacity for visual accommodation (Hughes, 1976).

Rabbits also possess stereoscopic vision (van Sluyters & Stewart, 1974; van Hof & Steele-Russell, 1977) but this has not been confirmed in rats (Wallace et al., 2013). The strength of the visual abilities in rabbits make their use as an animal model a better candidate to translate vision research to human and primate visual capabilities than comparison to a rat's ability.

PAVLOVIAN CONDITIONING PARADIGM

Nictitating membrane (NM) eye blink Pavlovian conditioning in rabbits is regarded as the most powerful animal model to study the cerebellum's role and neural pathways of Pavlovian conditioning and associative learning (G rmenzano, 1966). Before Pavlovian conditioning occurs, the NM eye blink in rabbits only naturally occurs in response to external stimulation such as physical contact or irritation. The eye blink does not occur spontaneously nor is it mediated by any instrumental behavior of the animal. This allows Pavlovian conditioning to be standardized across all animals and individual differences are minimized in both acquisition and performance of associative learning of the eye blink. As such, the experimenter has almost complete control over factors during associative learning.

The role of the cerebellar cortex in Pavlovian conditioning derives from the neural models of Marr (1969) and Albus (1971), who were the first to draw attention to the unique features of the cerebellar cortex's architecture - specifically the identical network connections throughout all cerebellar folia. They hypothesized that the cerebellar cortex played a major role in simple attentional learning such as Pavlovian conditioning. These neural models were further explored by Thompson (2005) and Thompson & Krupa (1994).

A critical axiom of current research on the cerebellar mechanisms of Pavlovian conditioning and learning is that there is a complete isomorphism between learning and the changes in the NM eye blink response (Thompson, 2005; Attwell et al., 2006;

Hesslow & Yeo, 2002). Lesions of the lobus simplex in the vermis of the cerebellum have long been reported to interfere with eye blink conditioning (Thompson & Krupa, 1994) due to purkinje cell activation in the lobus simplex during smooth pursuit eye movements indicating participation of the folium in oculomotor control (Suzuki, Koda, & Kase, 1981). Accordingly, the lobus simplex has been regarded not only as a region involved in attentional ocular movement control, but is also believed to be the storage site for Pavlovian conditioning.

This project literature survey will examine whether or not NM eye blink conditioning consists solely of cerebellar motor learning as indicated by the current Pavlovian neural models, in light of evidence indicating doubt on the cerebellum as the sole responsible cortical structure in NM eye blink reflex associate learning (Steele-Russell et al., 2008). The focus of this thesis project was the critical evaluation of evidence for an isomorphism between NM eye blink learning and changes in the significance and meaning of the NM eye blink response during Pavlovian conditioning across two discrete environments.

DESIGN

A repeated measures design was frequently employed where all subjects were exposed to the same battery of behavioral tests. In standard Pavlovian conditioning experiments, subjects are tightly confined within a Pavlovian stock (Figure 1) – an apparatus that permits very little behavior save the eye blink movement. It is very possible that this highly restrictive confinement is insensitive to the measurement and assessment of any additional changes in the subjects' behavior other than the expected eye blink during conditioning. For this reason, a transfer design was utilized wherein the S+ and S- signals were first presented in a Pavlovian stock, subsequently, the signals were then presented within an open environment to observe changes of behavior in reaction to the same signal presentations.

An operant conditioning chamber (Figure 2) served as the open environment and was utilized during the testing battery for continuous performance task testing (Castiglioni et al., 2009). The range of the subjects' reactions to presentations of the S+ or S- signals in the non-restrained open environment were recorded using a digital camcorder.

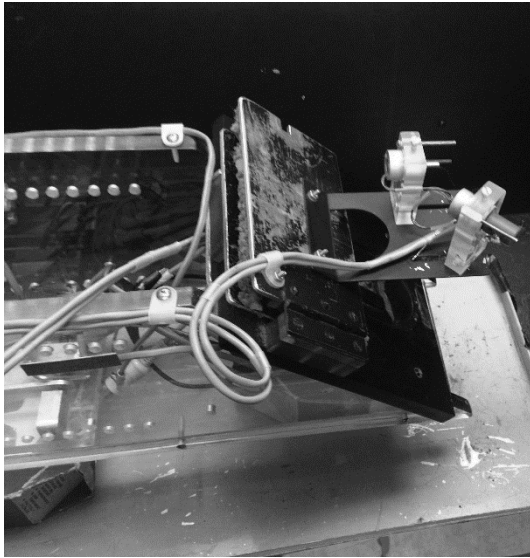


Figure 1. Pavlovian conditioning environment with restraining stock inside to restrain subject during conditioning.



Figure 2. Testing chamber for continuous performance task that served as open environment. Speakers located to the right and above the tunnel, transmit the S+ and S- signals. Tunnel with lever press located in the bottom left hand corner. Food cup located to the right of the tunnel distributed pellets after successful behavior.

Subjects were evaluated using an ordered behavioral test battery (Figure 3) to observing recordings of the subjects' behavioral reactions to presentations of the S+ and S- signals both before and after Pavlovian conditioning had occurred. The test battery included six test phases. The first, a continuous performance task (CPT), second, preconditioning sensory salience test (SST1), third, Pavlovian Conditioning (PavCond), fourth, an eye blink retention test (RT1), fifth, a postconditioning sensory salience test (SST2), and last, a second eye blink retention test (RT2).

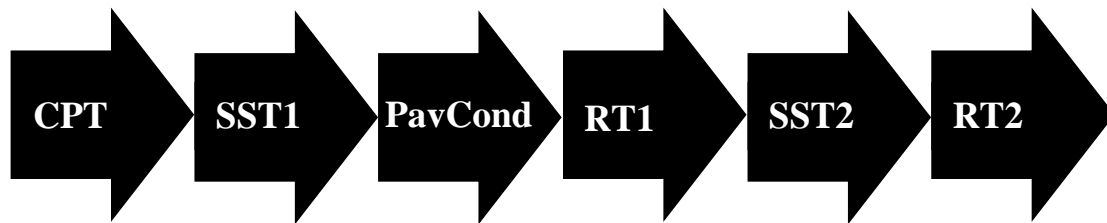


Figure 3. Sequence of experimental test phases in the testing battery.

CPT: Continuous Performance Task → SST1: preconditioning Sensory Salience Test → PavCond: Pavlovian Conditioning phase → RT1: first eye blink Retention Test → SST2: postconditioning Sensory Salience Test → RT2 → second eye blink Retention Test

CONTINUOUS PERFORMANCE TASK

All subjects were first trained in a continuous performance task (CPT) (Castiglioni et al., 2009), which was designed to measure changes in the salience of stimuli in open, non-restricted conditions before and after conditioning took place. At the same time, the task was designed to ensure rigorous psychophysical standards of sensory stimulation without denying the subject freedom of movement. The CPT is the most commonly used measure of attention in research, (Riccio et al., 2002) and is a free operant conditioned suppression task.

Subjects were placed into an open chamber and trained to perform a panel press response 15 consecutive times without delay to receive a food reward. Photosensors placed along the tunnel wall recorded the subjects' movements during their behavior to monitor any withdrawal when the distracting signals were presented and the response counter monitored any delay between panel presses (Castiglioni et al., 2009).

The CPT apparatus (see Figure 2), was first described by Werka et al. (1982) and Oakley & Russell (1977) and later modified per (Castiglioni et al., 2009) to include a push panel with an 8 cm tunnel projecting outward from the chamber's right wall. Every trial, subjects were required to press the panel 15 times rapidly in a row to terminate the trial and receive a food pellets as a reward. Both the initiation and termination of each trial was signaled by the tunnel light's onset and offset. A 1 second blackout period signaled the end of the trial. At stimuli presentation, subject distraction time were detected and recorded via the tunnel's infrared photosensors located laterally along both

tunnel walls. The photosensors measured subjects' retreat from the tunnel and reset the response counter to zero. Additionally, distraction times longer than 1 second, while subjects remained in the tunnel also reset the response counter to zero. The onset tunnel light presented after the previous trial's 1 second blackout, and remained on throughout the trial until subjects completed 15 panel presses to be rewarded with food pellet delivery.

Each testing session consisted of 100 responses. Subjects were trained until criterion was met – consisting of 5 continuous sessions with minimal spontaneous pauses, between each response cycles.

The modified apparatus' tunnel provided unique advantages over traditional CPT experiments. Firstly, it enabled rigorous psychophysical control over any background sensory stimuli outside of the S+ and S- . These background stimuli are typically unaccounted for and uncontrolled in CPT studies. All subjects had identical head and body orientations as they entered the tunnel to begin the panel presses where the speaker projected auditory stimuli from the same location towards the subjects' head and ears. The subjects' constant head positioning relative to the chamber's speakers controlled against any sound wave distortion.

Secondly, utilization of the light source – a panel located within the tunnel, ensured that visual stimuli indication for the trial beginning and termination, at which point in time the subject could then retrieve the food reward. The light panel was independent of the condition as it was a self-indexing stimulus. Thirdly, the tone and white noise signals utilized in the CPT were the same stimuli utilized during the

Pavlovian conditioning phase, ensuring the auditory signals across the testing battery were uniform.

Fourthly, throughout both SST phases, additional body movements by the subjects irrelevant to the learned behavior had no effect on the recorded distraction times as these were outside the tunnel and thus away from the panel. Often in free operant conditioning, such innocuous movements could mistakenly trigger the behavior for that particular conditioning. Lastly, the utilization of tunnel photosensors allowed precise recording of the subjects' withdrawal when presented with distracting signals during the performance of the CPT.

PRECONDITIONING SENSORY SALIENCE TEST (SST1)

Once subjects have mastered this operant conditioning behavior, subjects moved to the second phase in the testing battery, the Preconditioning sensory salience test or SST1. The SST1 utilized the CPT as a measure for the sensory salience of the S+ and S- auditory signals before Pavlovian conditioning occurred. At this stage, the signals hold no significance to the subjects as these are novel stimuli previously not encountered. For all subjects, the S+ or S- signals served as the distracting signals and were presented at the start of the thirtieth trial with the first panel press response over two sessions. Both the S+ and S- consisted of either a pure tone or white noise signal both at identical sound pressure levels. Both stimuli from onset remained on until subjects actively terminated either stimuli with the 15 panel press, also terminating the trial and producing the food reward and extinguishing the tunnel light. The duration of time between the signal onset and the period it took for subjects to complete the panel press were recorded as distraction times (Castiglioni et al., 2009).

PAVLOVIAN EYE BLINK CONDITIONING

Third in the test battery, subjects received Pavlovian conditioning in a separate sound insulated chamber, as described by Steele-Russell et al. (2006) and Castiglioni, et al. (2009). This chamber contained a restraining stock, with a head restraining device which was used to ensure that the subjects' position to the sound source was constant throughout the testing period. The subject in the stock was oriented such that they faced the front of the chamber, towards the speakers and light panels. Along this front wall, two audio speakers mounted vertically, and two light panels mounted horizontally. Two transdermal electrodes were placed within the periorbital cutaneous receptive field of the trigeminal nerve.

Using a different Pavlovian conditioning paradigm, the auditory S+ tone was paired with a cutaneous shock, the unconditioned stimulus (US). The S- white noise was not paired with the US. The auditory S+/S- stimuli sounded from a speaker placed along the end wall of the conditioning chamber 2 cm above the lateral visual.

Finally, the use of attached dermal electrodes in combination with a computer controlled shock (US) source ensured that all subjects received identical stimulation to the same location on their cutaneous receptive field of the trigeminal nerve. Each tonal S+ and S- signal, was 500 ms in duration. For S+ and US paired presentations, the US consisted of a 1.0 mA 50 Hz shock administered to periorbital receptive field for the remaining 200 ms of the CS. The end of the trial was signaled by the co-termination of the S+ and the US. This degree of psychophysical control is currently not available with

conventional foot shock apparatuses utilized in other conditioned suppression paradigms.

All subjects had to be first habituated within the restraining stock over four days without S+ or S- presentation, to habituation to the sensation of being restrained in the stock and to ambient sounds, electrode attachment, and ambient cues from within the chamber. Following habituation, subjects were trained daily in 40 minute sessions. The first and third of these sessions were sit-control treatments and the second and fourth sessions were conditioning treatments. In each sit-control session subjects were given identical treatment to the conditioning sessions with the exception that no S+ signals were presented. The sit-control treatments exposed subjects to any background stimuli inherent to the test chamber with no US presentations to direct subjects' attention to subsequent conditioning pairing with the S+. A total of ten presentations of the S- white noise signal were randomly presented throughout both sit-control sessions.

In each conditioning session, five S+ and US paired presentations occurred at variable intertrial intervals (ITI) of an average 180 seconds. The five S- signals had the same presentation time as the S+ signals, but were not paired with the US. For all subjects, the Pavlovian conditioning used the S+, a pure tone of 257 Hz, signal paired with the US, and the S-, white noise signal, not paired with the US. Both the S+ and S- signals were 75 dB to control against sound intensity differences. Across both training sessions, 10 S+ and US pairings were given with a variable ITI (180 seconds) to control against temporal condition. Subjects met criterion after five consecutive sessions when 80% or more of eye blink responses were conditioned responses.

EYE BLINK RETENTION TEST 1

On the day following the last conditioning session, all subjects were given a retention test of the conditioned eye blink response. This took place in the Pavlovian stock under identical conditions to the Pavlovian conditioning (dermal electrode placement, any ambient sounds, etc.). The tonal stimuli used were identical to the Pavlovian conditioning and previous Continuing Performance Task. The retention test consisted of a random mixture of 10 S+ and 10 S- signals presented singly on any trial under extinction conditions. Thus both S+ and S- signals were each presented alone without any pairing with the US. For both signals, behavior to the S+ and S- were recorded as the number of Hits: eye blink response to S+ presentation, or the number of False Alarms: eye blink response to S- presentation.

POSTCONDITIONING SENSORY SALIENCE TEST (SST2)

The fourth phase in the testing battery, again utilized the CPT as an independent measure of significance to the S+ and S- Pavlovian retention test. Subjects were retrained on the CPT for three sessions before the sensory salience tests (SST) were administered. After which, the same S+ and S- signals used in the Pavlovian Conditioning phase were presented as either a pure tone or white noise signals with identical sound pressure levels of 75 dB respectively. During this phase, there was no pairing of the US shock with the S+ or S-. On the beginning of the thirtieth trial, the S+ or S- signal was triggered by the first panel press and continuously played until subjects actively extinguished stimuli presentation with the 15 consecutive panel presses. This resulted in a food reward and also extinguished the tunnel light. The duration of time between the signal onset and the period it took for subjects to complete the panel press were recorded as distraction times.

Distraction times recorded after subjects completed Pavlovian conditioning during this salience test was referenced to the subjects' performance on the first SST (SST1) to the same stimuli. Any increase in distraction times can be attributed to conditioning altering the subjects' meaning and significance of the S+ and S- stimuli (Castiglioni et al., 2009).

EYE BLINK RETENTION TEST 2

Following the completing of the SST2, all animals were returned to the Pavlovian stock and tested for retention of the eye blink condition with only S+ presentations not paired with the US. This extinction procedures used after the second sensory salience test in the SST2 phase were identical to the procedure used in the first eye blink retention test. Eye blink responses to the S+ presentation were recorded as Hits, and eye blink responses to the S- presentation were recorded as False alarms.

RESULTS

Review of the video records revealed a typical subject response pattern to the S+ and S- signal presentation in the open environment after Pavlovian conditioning occurred. During Pavlovian conditioning, subjects only displayed the eye blink conditioned response to S+ signal presentation. After Pavlovian conditioning, during the SST2, subjects responded to the S+ onset with a significant distraction time, and copious emotional reactions (Castiglioni et al., 2009). The S- presentations in the Pavlovian stock produced no signs of any conditioning. S- presentations during the SST2 typically included a brief orientation to the source of the S- without any emotional reactions before continuing with the CPT.

DISCUSSION

The present review indicates that Pavlovian eye blink conditioning is more complex than the simple motor reflex learning that is central to current theoretical models of conditioning (Thompson, 2005; Hesslow & Yeo, 2002). The vast majority of Pavlovian work was only measured conditioning, which corresponds in our model to the second eye blink retention test during which the restricted conditions are applied and the subjects' overt behavioral repertoire is limited to an eye blink. To our knowledge, the present experiment is the first to have examined the effects of differential eye blink conditionings using both restraint and non-restraint test conditions. Furthermore, use of an intramodel auditory discrimination where the S+ tone and S- white noise had identical sound pressure levels energy levels to prevent any tempering of learning due to stimuli differences. This is an improvement on earlier studies by Oatley and Steele-Russell (1975, 1977) using extramodel stimuli of S+ light and S- sound where the differing energy levels give inherent salient differences between light and sound modalities.

The typical reaction of subjects when confronted with the S+ in the non-restrained environment of the CPT, was a hasty retreat withdrawing from the tunnel to the furthest wall of the CPT chamber. Subjects also responded to the stimuli presentation with bruxing, or teeth grinding, a typical aggression response in rabbits. After rapidly retreating, subjects made several attempts at reentering the tunnel, with several slight advancements and retreats from the tunnel. Returning to the tunnel was generally a slow and cautious approach, whereas all retreats were hasty withdrawals out of the tunnel. All

subjects displayed this same behavioral response with minor variation in the distraction times between subjects. No eye blink responses were seen in subjects when presented with the S+ only variations of the dominant reaction pattern of rapid withdrawal and aggressive responses. Yet, during the Pavlovian conditioning within the restrictive environment, the only observable behavior to UR and the S+ was the Nm eye blink response. There were no signs of emotional behavior such as struggling, vocalization, or bruxing.

Distraction times to the S+ and S- presentation in the SST1 were low to minimum as expected of novel stimuli in the preconditioning phase. After Pavlovian conditioning occurs, we see distraction times significantly rise for only the S+ presentations. The subjects have now learned to differentiate between the two signals, and displayed an emotionally charged retreat previously unseen in the Pavlovian stock. Distraction times to the S- signal in the SST2 (postconditioning) remained unchanged, and are identical from SST1 (preconditioning).

The first eye-blink retention test (RT1), given immediately following Pavlovian eye-blink conditioning showed that 78% of conditioned responses occurred to the S+ trials and 22% to the S- trials, which is evidence for specific conditioning to the S+ tone. The second eye-blink retention test was given after SST2 and results show both the S+ and S- signals were presented twice under extinction conditions where each subject was exposed to each signal until they actively terminated the signal by resuming work on the CPT.

In the second eye-blink retention test, both the S+ and S- signals had been exposed to extinction in the previous SST2 phase. When tested on the second eye-blink retention test of conditioning, there was a complete extinction of the former S+ responding. These findings indicate that there is bidirectional transfer of learning from the Pavlovian training to the SST, and from the SST to the Pavlovian environment.

At the conclusion of the testing battery, subjects underwent additional Pavlovian conditioning to examine any preferential conditioning to the usage of tone as the S+ in the experimental testing battery. Subjects were retrained in the Pavlovian stock with the same stimuli and shock parameters. Conditioning began with the tone stimuli paired with the US shock. Once criterion was met, a stimuli reversal was implemented. The white noise signal became the S+ paired with the US shock, and the tone signal became the S- paired with nothing. Subjects were again conditioned until criterion was met. A second reversal was then implemented, wherein the tone became S+, and the white noise became S-. These reversals served to indicate no inherent properties of the tone and white noise stimuli contributed no bias to the subjects' conditioning for the previously mentioned experimental testing battery as regardless of which auditory signal used (white noise or tone) as the S+, subjects conditioned. This suggests the experimental testing battery could alternatively have used the white noise as S+ and tone as S- and receive the same results.

CONCLUDING COMMENTS

In addition to the role of the cerebellum in retention of Pavlovian conditioning as a motor reflex controlled by cerebellar folia (Lavond, et al., 1981; McCormick, et al., 1981; McCormick, et al., 1982), this review provides a strong argument that Pavlovian conditioning involves the acquisition of a change in the meaning and emotional significance of the S+ stimulus. This is expressed in different situations by markedly different response patterns which unmasked a significant emotional component of the conditioning. Further, these results established that the emotional changes were exchanged bidirectionally between the very different test environments, seen in the subjects' changed responses to the S+ and S- in RT1 to RT2.

NM eye blink conditioning consists of more than a change in a single discrete cerebellar oculomotor reflex as shown by the Thompson (2005) and Hesslow and Yeo (2002) models. The clear emotional component to the conditioning indicates the involvement of extracerebellar control mechanisms in possibly the frontal cortex and amygdala.

Separate pathways to the cerebellar cortex are identified for the US and S+ inputs. The US pathway goes from the trigeminal nucleus to the inferior olivary nucleus via climbing fibers to the eye blink microzones in the lobus simplex. Different S+ pathways are used for the different senses of vision, audition, or tactile modalities. The S+ information is conveyed via mossy fibers from the appropriate precerebellar nucleus – in the present experiment the pontine nucleus for the auditory S+ channel – via parallel

fibers to Purkinje cells. A critical element in all cerebellar models of associative learning is that the cerebellum plasticity changes mediating condition are restricted solely in an invariant eye blink response.

It is important to note that the great majority of eye blink conditioning research uses such a single CS training paradigm. This has resulted in widespread failure to control for both the specificity of the CS learning for pseudoconditioning. Steele-Russell et al. (2006) showed that such single stimulus conditioning can be completely non specific with subjects responding to any stimulus change in the testing situation. The standard control to preclude the occurrence of pseudoconditioning is to use a differential conditioning paradigm. Previous studies (Oatley & Steele-Russell 1977) have used crossmodal comparisons confounded with salience as well as modality such as tone versus light stimuli. For example, a nocturnal animal would have greater sensitivity tuning for auditory signals than visual ones. Therefore, the present experiment used auditory intramodal comparisons between pure tone versus white noise matched for equal sound pressure levels.

Continued research will be needed to further investigate and isolate the role of extracerebellar structures in the emotional changes and learning in Pavlovian conditioning. Uncovering the neural pathways of Pavlovian conditioning in rabbits is a positive step in completing mapping of neural networks of the rabbit brain for learning and towards further developments of a rabbit animal model.

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